Cloning into the inducible vector pYES2

Introduction

After day 10 of cloning the codon optimized Subtilisin E into pTEF-MF we tried a parallel strategy in the PYES2 vector. A scheme of the cloning can be seen below:

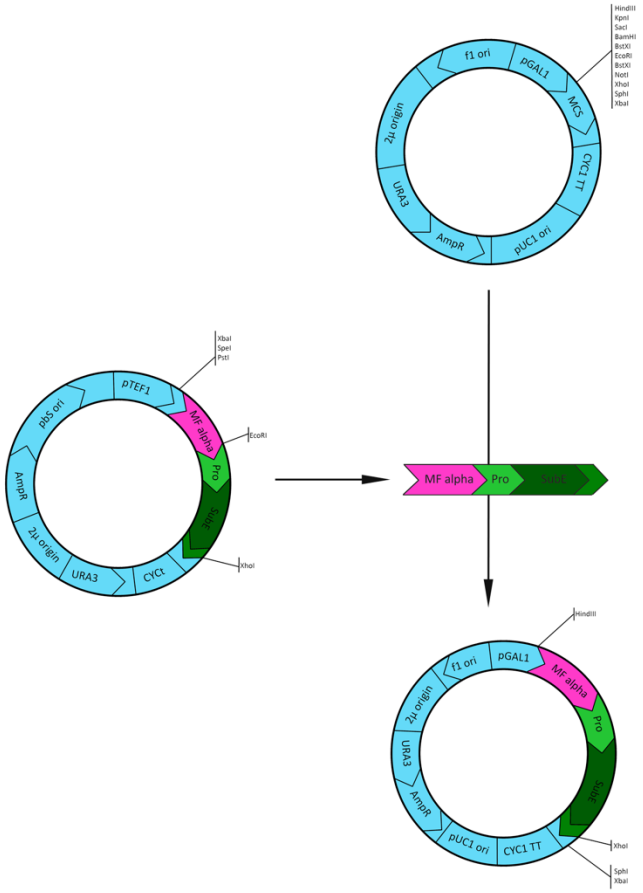


Figure 1: Scheme of cloning codon optimized Subtilisin E with MFalpha into the pYES2 vector

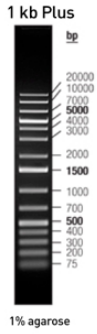


Figure 2: 1kb Thermo scientific ladder

For all agarose gels we used 1% agarose and 1 kb Thermo scientific ladder 08.

Day 1

PCR amplification of codon optimized Subtilisin E (cSubE) with MFalpha

As template we used the pTEF-MF with inserted codon optimized Subtilisin E gene. With primers YP0016 and YP0017 we amplified the Subtilisin E including the prefixed MFalpha which is on the pTEF-MF vector. These two Primers generate a HindIII overhang before the Subtilisin gene and a XhoI overhang after. The PCR was set as on day 9's colony PCR of cloning into pTEF-MF.

PCR Clean Up

We made a PCR clean up of the cSubE + MFalpha PCR samples in one tube collected.

Digestion

We double digested the cSubE + MFalpha PCR product and the pyes2 empty vector with HindIII and XhoI.

Dephosphorylation

We dephosphorylated the cut pyes2 backbone.

Ligation

We ligated the cSubE + MFalpha with the dephosphorylated pyes2:

Backbone pyes2 (200ng/µl)	1 µl
Insert cSubEMfalpha (211ng/µl)	2,5 µl
T4 ligase buffer	2 µl
T4 ligase	1,5 µl
ddH2O	13 µl

As a control for the dephosphorylation we self-ligated the dephosphorylated backbone.

Backbone pyes2 (200ng/µl)	3 µl
T4 ligase buffer	2 µl
T4 ligase	1,5 µl
ddH2O	13,5 µl

We left the ligation samples overnight at 16°C.

Day 2

Transformation in DH5alpha

We transformed yesterday's ligations into *E. coli* DH5alpha.

Day 3

Transformation Results

The transformation was successful. Colonies grew on all transformation plates. The negative control of dephosphorylated backbone ligation did not grow.

Overnights
We picked 5 colonies of the transformed cSubE + MFalpha in pyes2 (clone2) and made 5ml overnights in LB + Amp medium. They were labelled clone 2.1-2.5.

Day 4

Cryo Cultures
We made cryo cultures from overnight cultures of the clones 2.1-2.5.

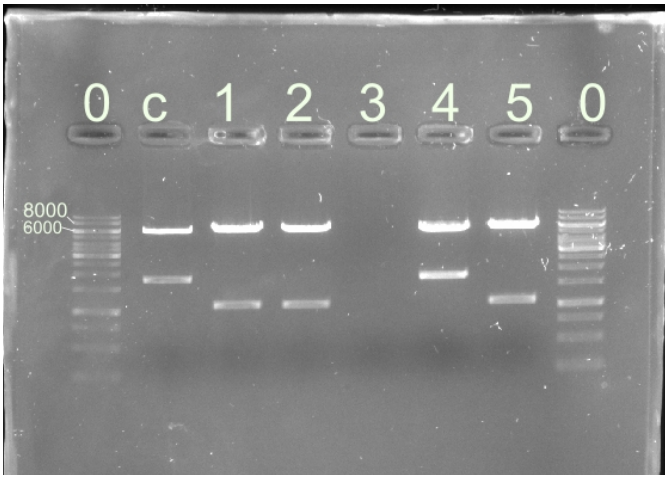
Plasmid isolation
We isolated the plasmids from the cells in the remaining overnight cultures.

Nanodrop
We measured the concentrations of the isolated cSubE + MFalpha in pyes2 plasmids:

2.1 180.5 ng/µl
2.2 778.7 ng/µl
2.3 616.3 ng/µl
2.4 648.3 ng/µl
2.5 686.3 ng/µl

Test Digestion
We double digested the isolated plasmids with EcoRI and XhoI to check their size on an agarose gel.

Agarose Gel
To see if the insert was cut out during the test restriction and if it has the right size, we run an agarose gel. the expected size of Subtilisin E insert is 1100bp.



C= control pyes2 with laccase insert (1600 bp)
1-5= 2.1-2.5 double digested with EcoRI and XhoI

- ⇨ C and 4 showed 1600bp insert, so these did not have the Subtilsin E + MF alpha insert
- ⇨ 3 is not visible on the gel

Sequencing
We sent clone 2.1,2.2 and 2.5 for sequencing with YP0019 and YP0030.

Day 5

Sequencing Results
Just the sequence of clone 2.1 showed a perfect alignment to cSubE and Mfalpha.

Transformation into *S. cerevisiae*
We transformed the plasmids of clone 2.1 into *S. Cerevisiae* CENPK2-1D. As control we transformed the pyes2 vector with laccase insert as we did not have an empty pyes2 vector.

Day 8

Transformation
The transformation was very ineffective. We had one colony of clone 2.1. The positive control had lots of colonies.

Overnights
We made an overnight of the colony in SD-U medium + Amp.

Results

After day 8 we were able to start the test expressions in *S. cerevisiae* CENPK2-1D.